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Matrix Metalloprotease-Sensitive Doxorubicin-Loaded Liposomes for Enhanced Anticancer Activity

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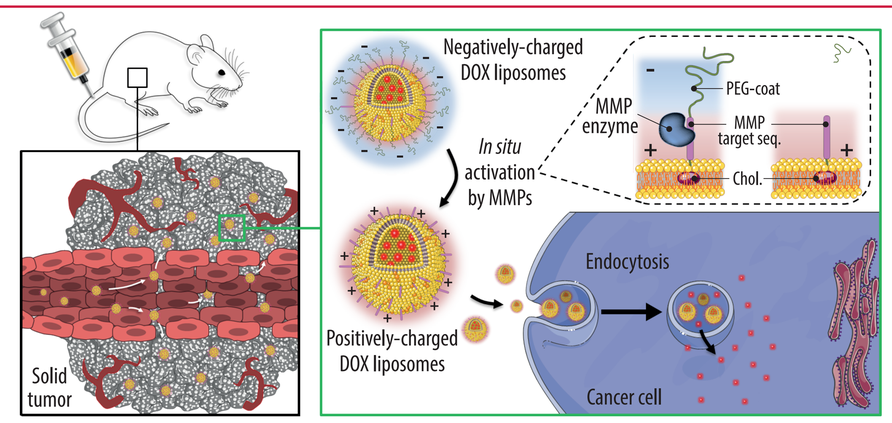
Purpouse

Long-circulating PEGylated liposomal formulations of doxorubicin (DOX) have shown to increase drug accumulation in tumors and reduce dose-limiting effects of DOX (e.g. cardiotoxicity); however these systems have only achieved a moderate improvement in anti-tumor activity due to poor cellular internalization and drug bioavailability[1]. We have recently developed an enzyme-sensitive liposome system that exploits the proteolytic action of matrix metalloproteases (MMPs), over-expressed in broad range of cancers, to produce the detachment of the PEG-coat and induce a membrane charge shift that will promote their internalization by tumor cells and the intracellular drug release[2]. We aim to demonstrate that DOX formulated into cationic liposomes covered with a detachable negatively-charged PEG-coating are efficient nanocarriers to enhance the delivery of DOX to tumor cells and improve the anti-tumor activity.



Results

The MMP-sensitive formulation increased the uptake of the liposomes by the cells upon proteolitic activation, resulting in an increased intracellular delivery of DOX and improved cytotoxic activity as compared with DOXIL (Fig. 1-3). The DOX-loaded MMP-sensitive liposomes showed a reduced blood circulation profile as compared with DOXIL (Fig. 4), suggesting a premature leakage of DOX from the carrier and/or fast removal by the RES. Thus, DOX levels found in the tumor were reduced whereas in the spleen were significatly higher. Encapsulation of DOX within MMP-sensitive liposomes resulted in slightly better tumor growth inhibition compared to the Free DOX but failed to provide similar therapeutic efficy as DOXIL.

In vitro cell uptake and growth inhibition of DOX formulated in MMP-sensitive liposomes

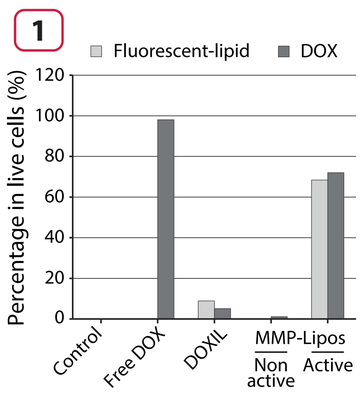


Figure 1. *In vitro* uptake of free DOX, DOXIL and DOX-loaded MMP-sensitive liposomes on CT26 cancer cells after 4 h incubation. FACS was used to detect the cell uptake of lipid DOPE-ATTO655 in FL6 (660±20nm) and DOX in FL2 (575±30nm).

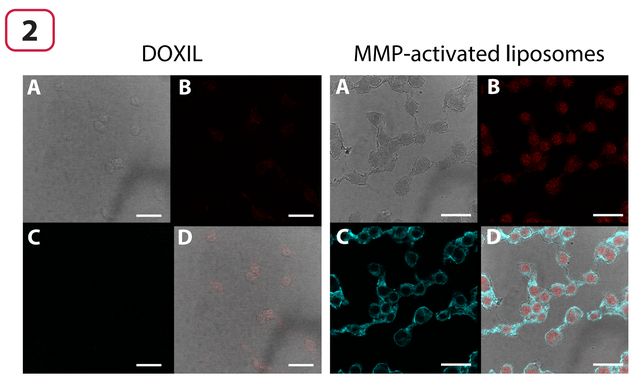


Figure 2. Confocal imaging of fixed CT26 cancer cells treated during 4 h with DOXIL or MMP-activated liposomes loaded with DOX. Both formulations included DOPE-ATTO655 (0.05mol%) as fluorescent label. The samples were excited with an argon (488 nm) and HeNe (633 nm) lasers. A) BF image; B) DOX signal (560-620nm); C) DOPE-ATTO655 signal (665-720nm); D) overlay image. Scale bars are 50 µm.

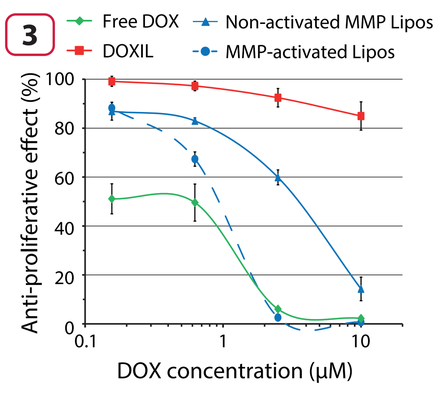


Figure 3. *In vitro* anti-proliferative effect of free DOX, DOXIL and DOX-loaded in MMP-sensitive liposomes either activated or not, on CT26 cancer cells. The cells were incubated with the compounds for 4 h, washed with complete medium and further incubated for additional 68 h. Then the proliferation was assessed by MTS assay.

In vivo evaluation of DOX-loaded MMP-sensitive liposomes

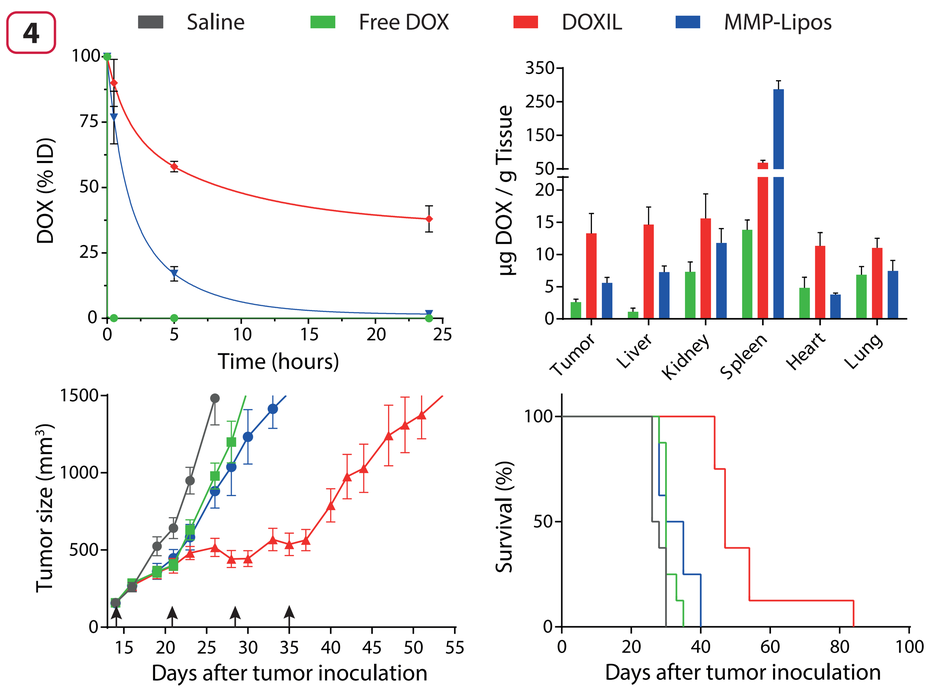


Figure 4. Pharmacokinetics and biodistribution (upper panel) of DOX-loaded MMP-sensitive liposomes. CT26 tumor-bearing Balb/c mice were injected with 10mg/kg of Free DOX, DOXIL and DOX-loaded MMP-sensitive liposomes. Blood samples were taken at 0.5 h, 5 h and 24 h, then the animals were sacrified and tissues were dissected. DOX was extracted following a acidified isopropanol extraction method[3] and detected fluorescently (ex.470nm/em.590nm). To assess the therapeutic efficacy of MMP-sensitive liposomes, tumor growth and survival rates (lower panel) were monitored after mice received 4 injections of 4mg/kg (qweek) of saline solution, Free DOX, DOXIL and DOX-loaded MMP-liposomes.

Conclusions

The results show the remarkable capacity of the MMP-triggered liposomes to increase the cellular internalization of DOX and efficiently improve the *in vitro* growth inhibition of cancer cells compared to DOXIL. However, the pharmacokinetic and biodistribution profiles demonstrated poor circulating properties and tumor accumulation, leading to an unimproved *in vivo* therapeutic potential compared to DOXIL. Further investigation on designing a more stable MMP-sensitive formulation should be carried on in order to ensure long circulating properties of the drug.

References

1. Barenholz Y. *J. Control. Release*. 2012 (160) 117-134.

2. Gjetting T., Jølck R., Andresen T. L. *Adv. Healthc. Mater.* 2014 (3) 1107-1118.

3. Laginha K. M., Verwoert S., Charrois G. J. R., Allen T. M. *Clin. Cancer Res.* 2005 (19) 6944-6949.

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